Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin S223P S65A

Enzyme description:- Parkin 1-465 (full length) S223P, S65A

Clone number:- DU39305

Source:- Recombinant

Tag:- cleaved from N-terminal His_6-SUMO-1

Purification method:- Ni^{++}-Sepharose, SEC

Expression level:- 1 mg/L

Calculated molecular mass:-

Monoisotopic	51600 Da
Average Mass	51633 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.21

Purity:- 95 %

Enzyme storage buffer:-

50 mM HEPES pH 8.2, 20% glycerol, 150mM NaCl, 0.5mM TCEP, 0.03% Brij35

Storage temperature:- -80ºC

Assay:-

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Clone Data Sheet

Protein name Parkin

Protein	Parkin 1 - 465 (full length) S223P S65A (rare variant)
Synonyms	PARK2, PRKN
Clone Number	DU39305
Species	Human
Accession Number	Protein: BAA25751
Tags	N-terminal His, followed by SUMO-1 to improve solubility
Aminoacid sequence of the expressed protein . SUMO-1 in grey, is removed during purification by SENP1 The final product, Parkin 1- 465 in bold	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTH LKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQE QTGG**MIVFVRFNSSHGFPVEVDSDTSIFQLKEVVAKRQGVPADQLRVIFA GKELRNDWTVQNCDLDQQAIVHIVQRPWRKGQEMNATGGDDPRNAAGGCE REPQSLTRVDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSF YVYCKGPCQRVQPGKLRVQCSTCRQATLTLTQGPSCWDDVLIPNRMSGEC QSPHCPGTSAEFFFKCGAHPTSDKETPVALHLIATNSRNITCITCTDVRS PVLVFQCNSRHVICLDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNS LIKELHHFRILGEEQYNRYQQYGAEECVLQMGGVLCPRPGCGAGLLPEPD QRKVTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDER AAEQARWEAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWC WNCGCEWNRVCMGDHWFDV**
Native sequence	in bold
Protease cleavage	SENP1 protease site underlined
Cloning sites	Complex cloning, please inquire.

DNA sequence of cassette

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCAACT
GAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAAGTCATT
GGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACAACACATCTC
AAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTTCCAATGAACTCA
CTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATACTCCAAAA
GAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAACAAACG
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